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INTRODUCTION

On July 21, 1914, some badly wilted nasturtiums (*Tropaeolum majus*) were received from Dr. John Arthur Luetscher of Baltimore, Md., who wrote concerning them:

> Seven years ago I raised a fine lot of nasturtiums, but in the last six years I have hardly been able to get a blossom, although the plants have been in the same soil and several times in the same plat. The leaves wither and the plant dies.

The plants, which were of the dwarf variety and much-branched, were poorly developed, and the leaves mostly wilted, yellowed, or dead (Pl. LXIII). The stems had a peculiar translucent or water-soaked appearance, allowing the vascular bundles to show as darkened streaks beneath the unbroken epidermis. When the stem was cut across, there oozed from these bundles a grayish white viscid slime which became brown on standing.

ISOLATION OF THE ORGANISM

On cross-sectioning such stems the vessels were found to be clogged with bacteria, often every bundle being entirely occluded. Agar-poured plates gave pure cultures of a white bacterial organism. Inoculations made from colonies on these plates into nasturtium stems produced signs of the disease—i. e., wilted leaves and water-soaked stems—within seven days (Pl. LXIV). From one of these stems the organism was reisolated on agar-poured plates and again produced typical wilt within four days when inoculated into healthy young nasturtiums, using subcultures from single colonies.

NATURE OF THE ORGANISM

Cultural work was then begun, but it was not until the growth on potato cylinders began to blacken that the identity of the organism with *Bacterium solanacearum* [1] was suspected. To test this hypothesis, inoculations were at once made into tomatoes (*Lycopersicon esculentum*), the only available plants being rather old. The result was the formation of numerous adventive roots in the vicinity of the needle pricks and the slow wilt of a few leaflets. Vessels were browned and filled with these

[1] Originally described by Erwin F. Smith as *Bacillus solanacearum* under the supposition that it was peritrichiate, but afterwards transferred by him to the genus Bacterium, in accordance with his system of nomenclature *in his* Bacteria in Relation to Plant Diseases. v. 1, p. 171. Washington, D. C., 1905. (Carnegie Inst. Wash. Pub. 27.)

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bacteria, as shown by microscopic examination and by poured plates. The plants then outgrew the disease. While not conclusive, these results did not contradict my supposition, since the organism plated from tobacco (*Nicotiana* spp.) and tomato often gives no more marked results when inoculated into old plants.

FURTHER CROSS-INOCULATIONS

A virulent strain of *B. solanacearum* obtained from tobacco from Creedmore, N. C., during the summer of 1914 was then available for comparison, and inoculations were made with this into nasturtiums of the tall variety by means of needle pricks from young agar subcultures. After 10 days all plants showed one or more wilted leaves and an abundance of the characteristic adventive roots near the point of inoculation (Pl. LXV, fig. 1). A month later one of these stems had produced adventive roots at intervals from 7 inches above the pricks to 20 inches below them, and in one case where the stem hung near the ground they were 3 inches long and had taken hold in the soil. Bacteria were present the entire length of the stem, which was now entirely leafless. Inoculations into dwarf nasturtiums produced a more rapid wilt but no adventive roots.

On young tobacco, prick inoculations with the nasturtium organism caused in five days an internal dark streak (visible on the surface) running several inches up and down the stem from the point of inoculation and the wilt of one or two leaves, but the plants always recovered. Check pricks produced no effect.

Inoculations with the nasturtium organism into *very young* tomato plants resulted in the rapid and complete wilt of the plants (Pl. LXVI, fig. 1). The entire vascular system became gorged with bacteria. Poured plates gave pure cultures of *Bacterium solanacearum*, as determined by cultures on typical media and by successful reinoculations into both tomato and tobacco plants.

TESTS ON OTHER PLANTS

A variety of plants were tested for susceptibility. Prick inoculations were made with both the Creedmore tobacco organism and the nasturtium organism into pelargoniums, soy beans (*Glycine hispida*), and lettuce (*Lactuca sativa*), all with negative results. Owing to the fact that Honing [1] in Sumatra has reported this disease on several composites and in young teak trees (Verbenaceae), inoculations were made on hothouse ageratum and on common cultivated verbena. Both became diseased but rather slowly. After 10 days the ageratum showed distortion of the leaves, one half being paler and smaller than the other, and after

[1] Honing, J. A., Een geval van slijmziekte in de djattibibit. Meded. Deli Proefstat. te Medan, Jrg. 7, Afl. 1, p. 12-15, also Naschrift, p. 59, 1912. See review in Smith, Erwin F., Bacteria in relation to plant diseases, v. 3, p. 254.

15 days complete wilt of several leaves occurred. The results were checked under the microscope. For the most part the plants outgrew the disease. Verbenas showed wilt within two weeks, and after three weeks the tips, as well as the leaves, for 2 inches below the point of inoculation, were completely wilted. Agar-poured plates from one of these stems gave pure cultures of *B. solanacearum*.

NATURAL METHODS OF INFECTION

The organism may enter the nasturtium plant through wounded roots or shoots or through the stomata. To demonstrate root infection, six nasturtium seeds were planted in each of four pots. Two pots were watered with a suspension from young agar cultures of the nasturtium organism and then covered with fresh soil. The others were held as checks. When the plants had four good leaves, the soil was worked in all the pots deeply enough to break some roots. Six weeks later one plant in an inoculated pot was badly wilted, and 10 days later four others succumbed, while those in the check pots were perfectly healthy. One week later all but 3 of the 12 plants in the inoculated pots had succumbed. Sections from stems of a number of these wilting plants were examined under the microscope. The vessels were seen to be clogged with bacteria, and there was the usual tissue disorganization.

Before the organism was identified, spray experiments were started to determine whether stomatal infections could be obtained. Well-grown plants of both tall and dwarf varieties of nasturtiums were sprayed in cages with a suspension from 3-day agar-slant cultures. Repeated spraying with sterile water kept the plants moist for 30 hours, after which they were removed from the cages. Six days later a few minute brownish spots appeared on the leaves, but these did not enlarge materially. Four weeks later, however, one plant was characteristically wilted, and within three weeks from this time all of the dwarf plants and one of the tall ones had succumbed, with characteristic bacterial infection of the vascular system.

Another experiment was made with young plants, each bearing two large leaves. They were sprayed in cages with suspensions from young agar cultures and kept moist for 48 hours. Four days after the experiment was started the large leaves all showed decided brown spotting and water-soaked areas. The spots centered about the stomata in every case examined, and often, but not always, they were marginal. After two weeks the spots had coalesced in many places and appeared to be affecting the small veins of the blade. Poured plates from such spots gave typical colonies of *B. solanacearum*. Portions of the leaves in both these stages were embedded, sectioned, and stained, and bacterial foci found in the substomatic chamber, thus demonstrating stomatal infection. In the younger stages the bacteria appear in the stomatal opening, as well as in the large chamber beneath (fig. 1). In older stages the

collapsed parenchyma is evidence of their presence, and careful search finds them lying closely appressed to the cell walls when they are not abundant in the intercellular spaces. In this stage they are also in the neighboring vessels of the leaf (fig. 2). In stained sections the walls of the vessels often show injury by taking a deeper stain than normal ones, even when the bacteria do not appear to have penetrated to their interior.

One leaf, which showed browning of some of the smaller veins, was sectioned at various points on the veins and petiole. The bacteria were numerous in the vessels of the browned area and for some distance below, but thinned out downward so that none were found in the base of the petiole or in the stem of the plant. In another case the bacteria

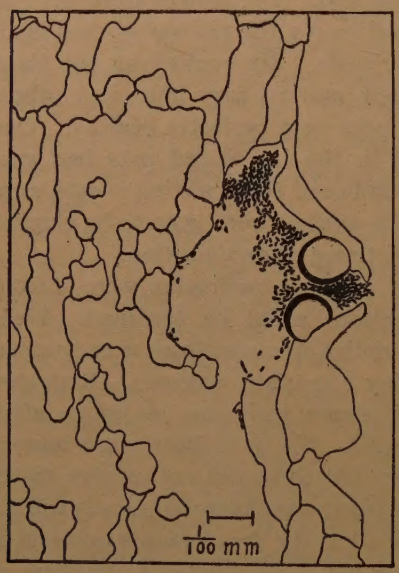

FIG. 1.—Section of nasturtium leaf four days after spraying with suspension of *Bacterium solanacearum*. Serially adjacent sections show bacteria throughout the substomatic chamber.

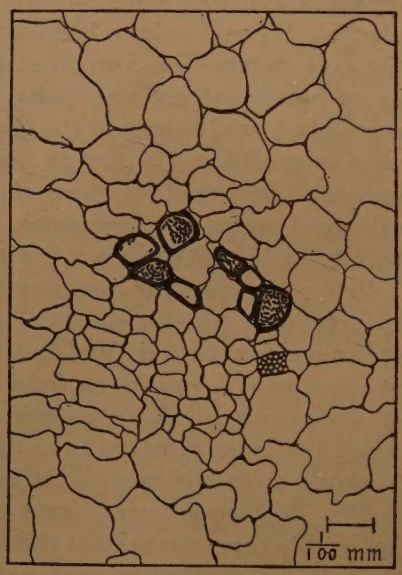

FIG. 2.—Cross section of a vein of nasturtium leaf, showing vascular infection nine days after spraying with suspension of *Bacterium solanacearum*.

were traced in the vessels all the way from the wilting leaf blade to the stem of the plant. Three plants in this set finally wilted completely. An early stage of vascular occlusion and cavity formation in the stem of a nasturtium, like that shown in Plate LXIII, is illustrated in figure 3.

Several attempts to produce stomatal infection on tomatoes and tobacco were made, but without success.

SUSCEPTIBILITY OF THE NASTURTIUM

From comparative needle-prick inoculations on nasturtium, tomato, and tobacco with the Creedmore tobacco organism, which was beginning to lose its virulence, it would appear that the nasturtium is very susceptible to infection by *B. solanacearum*, since it wilted readily, while

tobacco and tomato, except when very young, wilted only slightly and recovered quickly. The converse of this experiment led to the same conclusion—i. e., that the nasturtium is more susceptible than the tomato or tobacco—because the organism isolated from the nasturtium was more infectious to the nasturtium than to tobacco or tomato. Possibly the susceptibility of nasturtium may be due to the great succulence of the nasturtium stems. The Medan (Sumatra) tobacco organism,

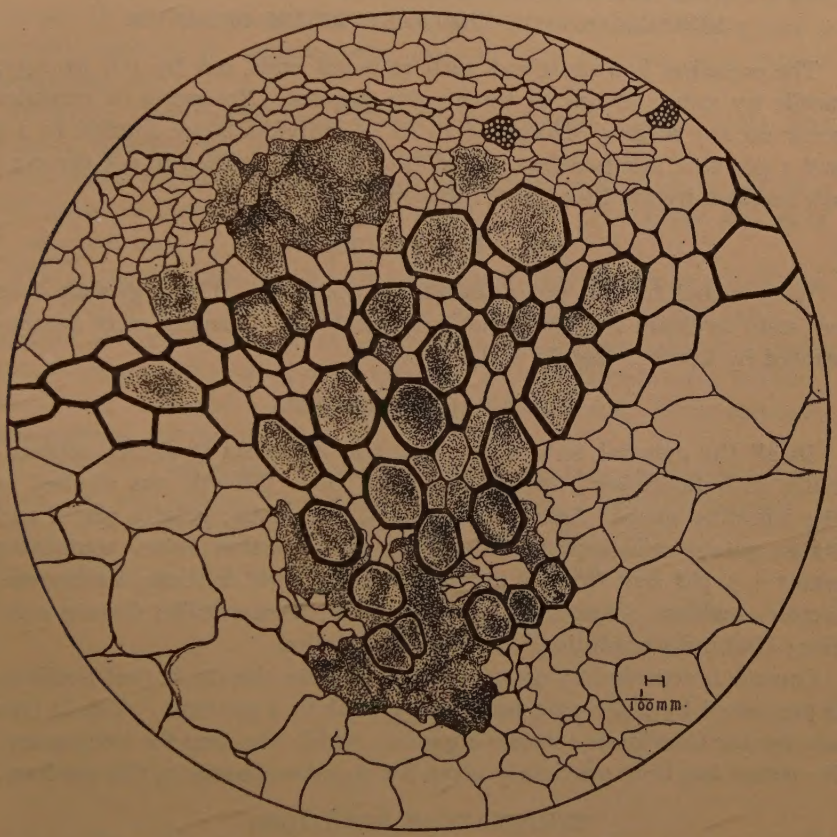

FIG. 3.—Cross section of stem of one of the infected nasturtiums from Baltimore, Md., showing the bacterial invasion of a bundle with the beginning of bacterial cavities. Two sieve plates are visible in the center phloem.

which had been extremely virulent but had been on media for a longer time than the Creedmore organism, was not able to infect either nasturtiums or tomatoes.

EFFECT ON THE TISSUES

The more tender parts of badly diseased plants become so translucent that the occluded browned vessels may be seen clearly through the water-soaked but unshriveled parenchyma (Pl. LXVI, fig. 3). In other cases the course of the affected bundle or bundles is marked superfi-

cially by sunken, reddish brown streaks or patches (Pl. LXV, fig. 2). Generally in the case of prick inoculations on nasturtiums of the tall variety adventive roots are formed at various points on the stem. These remain rudimentary except where the stems are near the ground, when they may become functioning roots. Check pricks failed to produce any root formation. No adventive roots occurred on any of the inoculated plants of the dwarf variety.

MORPHOLOGY OF THE NASTURTIUM ORGANISM

The organism is a short rod with rounded ends, 0.6 by 0.8 to 1.3μ, motile by means of one to three polar flagella. No spores or capsules occur on any media. Chains of 10 to 15 individuals are formed in 0.5 and 1 per cent salt bouillon. Similar chains are formed in 0.5 per cent salt bouillon by the Creedmore tobacco organism.

STAINING REACTIONS

With carbol fuchsin polar staining is obtained. The organism does not stain by Gram's method and is not acid-fast. Flagella were demonstrated by Löwitt's flagella stain.

CULTURAL CHARACTERS

In all the cultural tests made with this organism it agrees substantially with *Bacterium solanacearum* Erw. Sm. Growth was studied in the following media: Agar plates, slants, and stabs; gelatin plates and stabs; potato cylinders; beef bouillon; fermentation tubes containing water + 1 per cent Witte's peptone + 1 per cent dextrose, saccharose, lactose, maltose, mannit, or glycerin; milk; litmus milk; Cohn's solution; Uschinsky's solution; and Fermi's solution.

Growth is retarded by 0.5 per cent of sodium chlorid in beef bouillon, is prevented by 2 per cent, and is very weak in 1 per cent. This is true also for the Creedmore tobacco organism, which was used for comparison. No record has been previously given for *B. solanacearum* in this medium.

TEMPERATURE RELATIONS

The optimum for growth is about 30° C. No growth occurs at 39° C., very weak growth at 12° C., and none at 10° C. The thermal death-point lies between 48° and 52° C.

DESICCATION OF THE ORGANISM

When dried on sterile covers from young peptone-beef-bouillon cultures and kept in the dark at room temperature (21° C.), most covers gave growth after 24 hours when dropped into suitable bouillon, very few after 2 days', and none after 3 days' drying. The bacteria from 24-hour bouillon cultures were more sensitive to drying than those from 8-day-old cultures.

SUMMARY

The nasturtium is subject to a bacterial wilt disease, observed for the first time in the summer of 1914, which prevents blossoming, stunts the plants, and finally kills them. It is caused by a bacterium that in all morphological, cultural, and infectious characters agrees with *Bacterium solanacearum* Erw. Sm.

Cross-inoculations on the tomato and tobacco produced successful and typical wilt of these plants, while inoculations on the nasturtium with a virulent strain of *B. solanacearum*, isolated from tobacco, gave typical nasturtium wilt.

Infection takes place from infected soil through broken roots, but stomatal infection has also been demonstrated.

Cultivated ageratums and verbenas were found susceptible to infection with both the nasturtium and the Creedmore (N. C.) tobacco strains of *B. solanacearum*.

This paper adds another family to those already known to be subject to *B. solanacearum*. Described from the tomato, the potato, and the eggplant in 1896 by Dr. Erwin F. Smith,[1] this organism has now been proved infectious to one or more species of each of the following families: Solanaceae, Compositae, Leguminosae, Verbenaceae, Euphorbiaceae, Bignoniaceae, and Geraniaceae.

If tomatoes, eggplants, peppers, potatoes, peanuts, or tobacco have shown this wilt disease, they should not be followed by nasturtiums.

[1] Smith, Erwin F. A bacterial disease of the tomato, eggplant, and Irish potato (Bacillus solanacearum, n. sp.). U. S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 12, 28 p., 2 pl. (1 col.). 1896.

PLATE LXIII

Bacterially wilted nasturtium plant from Baltimore, Md.

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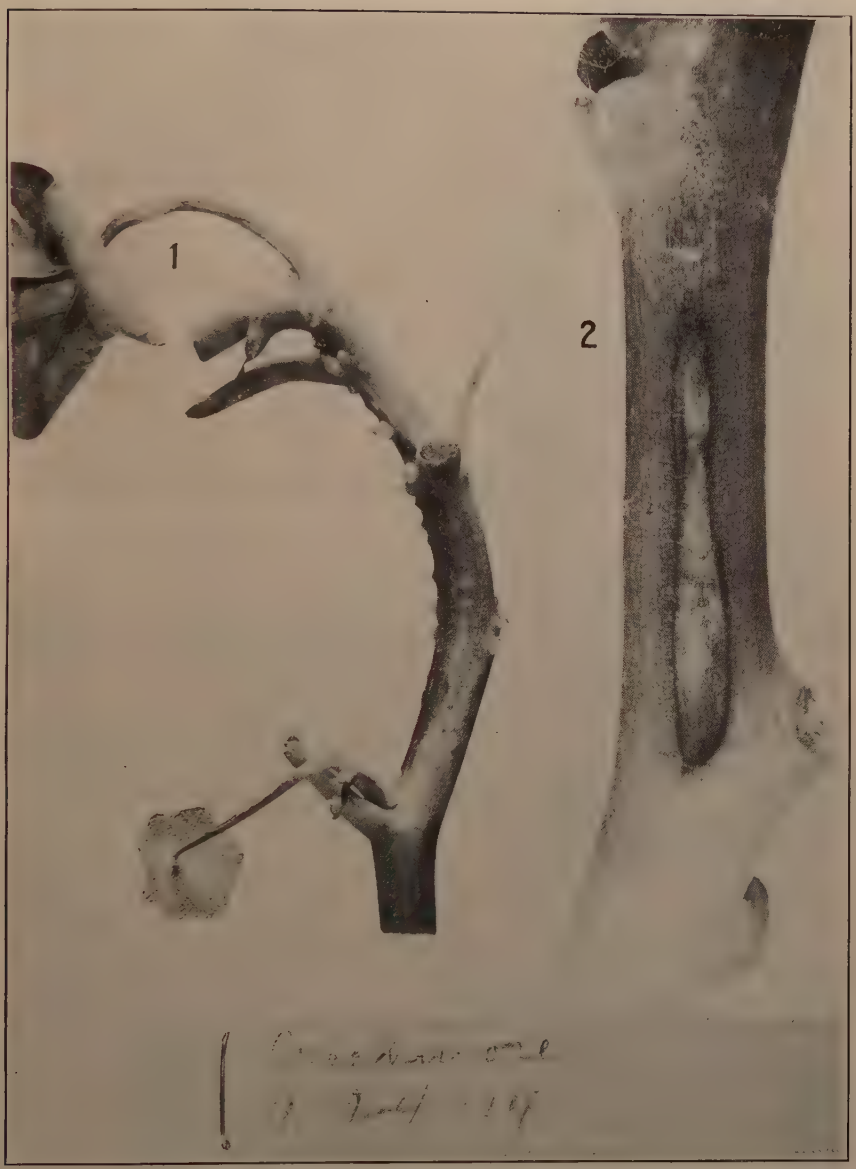
PLATE LXIV

Nasturtium plants four days after inoculation by needle pricks on the stem, using a pure culture of the bacteria cultivated from a plant infected like that shown in Plate LXIII.

PLATE LXV

Fig. 1.—Nasturtium plant (tall variety) 13 days after inoculation with *Bacterium solanacearum* from Creedmore (N. C.) tobacco, showing wilt of the foliage and development of roots near needle pricks.

Fig. 2.—Stem of a nasturtium plant inoculated with *Bacterium solanacearum* from tobacco, showing dark sunken stripe following line of infected bundle.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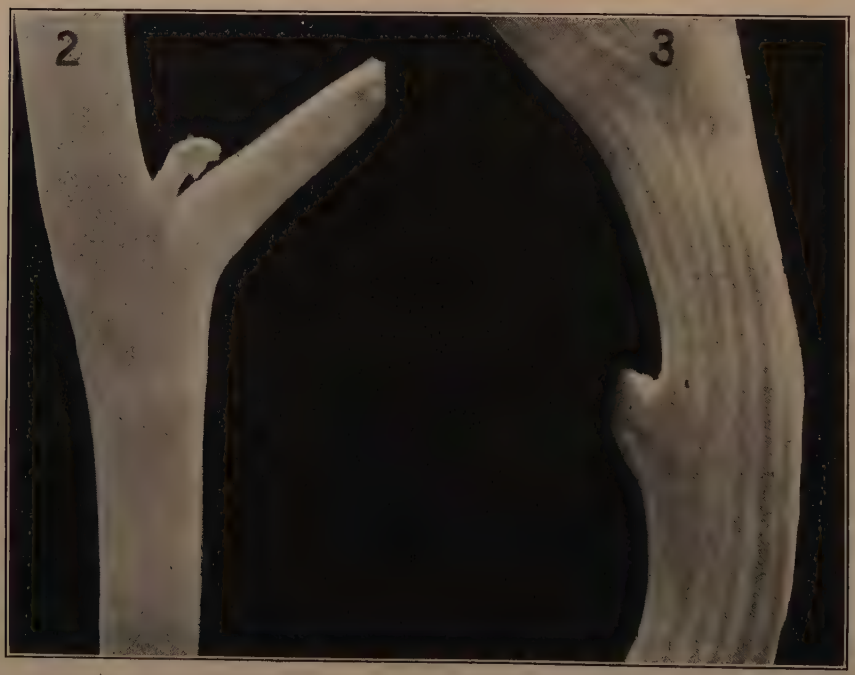
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PLATE LXVI

Fig. 1.—Young tomato plants six days after inoculation by needle pricks with the nasturtium organism, *Bacterium solanacearum*.

Fig. 2.—Normal nasturtium stem enlarged to show uniform (unstriped) appearance.

Fig. 3.—A nasturtium stem inoculated with *Bacterium solanacearum*, showing striping due to bacterial invasion of the bundles.